

---

# Human Beta-Endorphin, $\beta$ -EP ELISA kit

Catalog No. E0806h

(96 tests)

Operating instruction

**EIAab™**

[www.eiaab.com](http://www.eiaab.com)

---

**FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!  
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!**

## Intended use

This immunoassay kit allows for the use in vitro quantitative determination of human Beta-Endorphin,  $\beta$ -EP concentrations in cell culture supernates, serum, plasma and other biological fluids.

## Introduction

Endorphins are endogenous opioid biochemical compounds. They are polypeptides produced by the pituitary gland and the hypothalamus in vertebrates, and they resemble the opiates in their abilities to produce analgesia and a sense of well-being. In other words, they might work as "natural pain killers." Using drugs may increase the effects of the endorphins. Beta-Endorphin is an endorphin produced by the pituitary gland that is a potent pain suppressant.

Beta-endorphin is released into the blood (from the pituitary gland) and into the spinal cord and brain from hypothalamic neurons. The beta-endorphin that is released into the blood cannot enter the brain in large quantities because of the blood-brain barrier. The physiological importance of the beta-endorphin that can be measured in the blood is far from clear: beta-endorphin is a cleavage product of POMC which is the precursor hormone for adrenocorticotrophic hormone (ACTH), so it will be released whenever ACTH is released. The behavioural effects of beta-endorphin are exerted by its actions in the brain and spinal cord, and probably the hypothalamic neurons are the major source of beta-endorphin at these sites. In situations where the level of ACTH is increased (e.g. Addison disease), the level of endorphins also increases slightly.

Beta-endorphin has the highest affinity for the  $\mu$ 1-opioid receptor, slightly lower affinity for the  $\mu$ 2- and  $\delta$ -opioid receptors and low affinity for the  $\kappa$ 1-opioid receptors.  $\mu$ -receptors are the main receptor through which morphine acts. Classically,  $\mu$ -receptors are presynaptic, and inhibit neurotransmitter release; through this mechanism, they inhibit the release of the inhibitory neurotransmitter GABA, and disinhibit the dopamine pathways, causing more dopamine to be released. By hijacking this process, exogenous opioids cause inappropriate dopamine release, and lead to aberrant synaptic plasticity which causes addiction. Opioid receptors have many other and more important roles in the brain and periphery however, modulating pain, cardiac, gastric and vascular function as well as possibly panic and satiation, and receptors are often found at postsynaptic locations as well as presynaptically.

## Test principle

The microtiter plate provided in this kit has been pre-coated with an antibody specific to  $\beta$ -EP. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for  $\beta$ -EP and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain  $\beta$ -EP, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm  $\pm$  2 nm. The concentration of  $\beta$ -EP in the samples is then determined by comparing the O.D. of the samples to the standard curve.

### Materials and components

| Reagent                           | Quantity  |
|-----------------------------------|-----------|
| Assay plate                       | 1         |
| Standard                          | 2         |
| Sample Diluent                    | 1 x 20ml  |
| Assay Diluent A                   | 1 x 10ml  |
| Assay Diluent B                   | 1 x 10ml  |
| Detection Reagent A               | 1 x 120ul |
| Detection Reagent B               | 1 x 120ul |
| Wash Buffer<br>(25 x concentrate) | 1 x 30ml  |
| Substrate                         | 1 x 10ml  |
| Stop Solution                     | 1 x 10ml  |
| Plate sealer for 96 wells         | 1 x 5     |

### Sample collection and storage

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20° C or -80° C.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8° C within 30 minutes of collection. Store samples at -20° C or -80° C. Avoid repeated freeze-thaw cycles.

**Cell culture supernates and other biological fluids** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20° C or -80° C. Avoid repeated freeze-thaw cycles.

**Note:** Serum, plasma, and cell culture supernatant samples to be used within 7 days may be stored at 2-8 ° C, otherwise samples must stored at -20° C ( $\leq$  3 months) or -80° C ( $\leq$  6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature.

It is recommended that all samples be assayed in duplicate.

DO NOT USE HEAT-TREATED SPECIMENS.

### Limitations of the procedure

**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

Reagent preparation

**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

**Standard** - Reconstitute the **Standard** with 1.0 mL of **Sample Diluent**. This reconstitution produces a stock solution of 2,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The diluted standard serves as the high standard (1,000 pg/mL). The **Sample Diluent** serves as the zero standard (0 pg/mL).

**Detection Reagent A and B** - Dilute to the working concentration specified on the vial label using **Assay Diluent A and B** (1:100), respectively.

Assay procedure

Allow all reagents to reach room temperature. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Arrange and label required number of strips. Prepare all reagents, working standards and samples as directed in the previous sections.

1. Add 100 uL of **Standard**, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37° C.
2. Remove the liquid of each well, don't wash.
3. Add 100 uL of **Detection Reagent A** working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37°C. **Detection Reagent A** working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 uL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 uL of **Detection Reagent B** working solution to each well. Cover with a new Plate sealer. Incubate for 1 hours at 37° C.
6. Repeat the aspiration/wash as in step 4.
7. Add 90 uL of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate within 30 minutes at 37°C. Protect from light.

8. Add 50 uL of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

### Specificity

This assay recognizes recombinant and natural human  $\beta$ -EP. No significant cross-reactivity or interference was observed.

### Sensitivity

The minimum detectable dose of human  $\beta$ -EP is typically less than 3.9 pg/mL.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.

### Detection Range

15.6-1,000 pg/mL. The standard curve concentrations used for the ELISA's were 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, 15.6 pg/mL.

### Important Note:

1. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of all standards and specimens, although not required, is recommended.
5. When mixing or reconstituting protein solutions, always avoid foaming.
6. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
7. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
8. Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.

### Calculation of results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the  $\beta$ -EP concentrations versus the log of the O.D. and the

best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

#### Storage of test kits and instrumentation

1. Unopened test kits should be stored referring to the package label for frequent use, and stored at  $-20^{\circ}\text{C}$  for long time storage. The microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at  $2-8^{\circ}\text{C}$  in their pouch with the desiccant provided.
4. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.
5. Use fresh disposable pipette tips for each transfer to avoid contamination.
6. Substrate Solution is easily contaminated. If bluish prior to use, do not use.

#### Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.